The Estimation of Decimal Reduction Times

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The heat resistance of a batch of microorganisms exposed at a particular lethal temperature frequently is expressed by a parameter D (or Z) that denotes the time in minutes required to reduce the number of viable organisms present at any time by 90 per cent. Use of this parameter rests on the negative exponential survival rule that is observed to hold at least approximately; that is, on

$$N = N_0 \cdot 10^{-(t/D)} = N_0 e^{-kt} \tag{1}$$

where N_0 is the number of viable organisms at zero time, N is the number surviving at time t (in minutes), and e is the base of natural logarithms $(2.718\cdots)$. The decimal reduction time D is related to the survival-rate parameter k by

$$D = \log_e 10/k = 2.3026/k. \tag{2}$$

N frequently is estimated by a binomial count; that is, by the numbers of sterile and viable samples in groups that are exposed for various periods at the lethal temperature. An estimate of N, $\hat{N}$, can be calculated for a particular period by the relationship

$$\hat{N}_i = \log_e (n_i/r_i) \tag{3}$$

where n_i is the number of samples for the i'th period that are incubated and r_i is the number that prove to be sterile. Stumbo *et al.* (1950) justified the use of (3) by reference to the Halvorson-Ziegler formula for the dilution count, which is identical with (3). Both applications rest on the Poisson probability distribution for events that occur with low frequencies and thus (3) is valid for the small proportions of organisms that survive a period of severe heating as well as for a conventional dilution count. Stumbo *et al.* suggested that values for D be calculated by (3) for each heating period that achieved sterilization of some but not all of the samples, and averaged to give an estimate of D, $\hat{D}_{unw}$. At any given period

$$\hat{D}_i = \frac{t_i}{\log_{10} N_0 - \log_{10} \log_e \left(\frac{n_i}{r_i}\right)} = \frac{2.3026\, t_i}{\log_e N_0 - \log_e \log_e \left(\frac{n_i}{r_i}\right)} \tag{4}$$

Then

$$\hat{D}_{unw} = \frac{1}{j} \sum_{i=1}^{j} D_i \tag{5}$$

where j is the number of periods calculated. This method of estimation may be called the "unweighted average D for individual times" method or, more simply, the "unweighted average" method.

D values calculated by this method were shown by Reynolds and Lichtenstein (1952) to increase with increasing periods of heating, and the effect was investigated further by Pflug and Esselen (1954). This effect appears to cast doubt on our understanding of the survival-time relationship in the exposure of bacterial spores to lethal heat; thus, Reynolds and Lichtenstein suggested that the effect gives evidence against an exponential survival relationship, and Pflug and Esselen referred in general terms to a discrepancy between "quantitative counting" (plate counts?) and $\hat{N}$.

It is shown in this paper that the effect arises from a systematic bias in the unweighted average method of estimation. Unbiased methods are illustrated for the estimation of D values where binomial counts are used.

The Probability Distribution of Sterile Samples

Let P be the probability that a particular sample will be sterile; it will be related to the period of heating. The probability that a particular organism will survive being $\Pi = N/N_0$, the probability that it will not survive in this sample is

$$1 - \frac{N}{N_0} = 1 - e^{-kt} \tag{6}$$

by (1); therefore the probability that none of the N_0 organisms will survive (which is the probability of a sterile sample) is

$$P = \left(1 - \frac{N}{N_0}\right)^{N_0} = (1 - e^{-kt})^{N_0}. \tag{7}$$

Then

$$\log_e P = N_0 \log_e (1 - e^{-kt}) \tag{8}$$

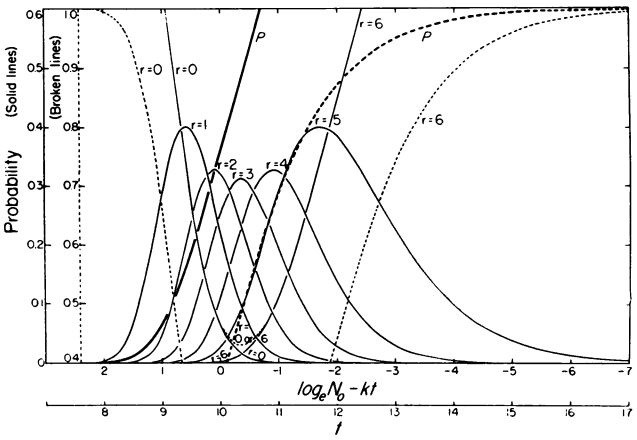

FIG. 1. Probability distribution curves for $r = 0$ to $r = 6$ sterile samples in groups of six, where the probability P of an individual sample being sterile is given by

$$\log_e(-\log_e P) = \log_e N_0 - kt,\ N_0 = e^{10},\ k = 1.$$

and if e^{-kt} is small, as it is when N_0 is large compared to N (by (1)),

$$\log_e P = -N_0 e^{-kt} \tag{9}$$

which is the approximation obtained by substituting $-e^{-kt}$ for x in the first term of the well-known series

$$\log_e(1 + x) = x - \frac{x^2}{2} + \frac{x^3}{3} - \cdots, -1 < x < 1. \tag{10}$$

Ordinarily only the first term will be significant; a borderline example will be illustrated later (in footnote 1 of Table 6). From (9) and (1)

$$P = e^{-N_0 e^{-kt}} = e^{-N} \tag{11}$$

Inasmuch as $p = r/n$ is an estimate of P

$$\hat{N} = \log_e (n/r)$$

which is equation (3). Furthermore $P = e^{-N}$ is the term of the Poisson probability distribution for zero occurrences where N is the expected or mean number of occurrences (surviving organisms).

The probability of obtaining r sterile samples in a group of size n, is given by the general term of the binomial probability distribution,

$$Pr\{r\} = \frac{n!}{r!(n - r)!} P^r(1 - P)^{n-r}. \tag{12}$$

It is apparent that $Pr\{r_i\}$, the probability of obtaining a particular value of r, will depend on r_i, n, and through (11) on N_0, t, and k or D.

DEMONSTRATION OF BIAS IN THE UNWEIGHTED AVERAGE METHOD

As an arbitrary example to illustrate the bias one may take $\log_e N_0 = 10$ (that is, $N_0 = e^{10} = 22{,}070$) and $k = 1$ (that is, $D = 2.3026$). Variations in k involve only the units of the time scale, and variations in N_0 (except to small values) involve only the displacement of the critical range of sterilization on the time scale (figure 1). One might calculate P for various values of t by (11) as a preliminary to calculating $Pr\{r\}$ by (12). More conveniently, one may take arbitrary values of P and calculate the corresponding values of t and $Pr\{r\}$. Tables of the latter have been published (National Bureau of Standards, 1950; Harvard Computation Laboratory, 1955). The results of such a series of calculations for $n = 6$ are shown in figure 1. The asymmetrical character of all of the curves may be noted.

TABLE 1. *Possible values of D of $t = 13.20$, $N_0 = e^{10}$, $k = 1$, $n = 6$ ($P = 0.96$), and the weighted average or expected value, D_{exp}*

r	$Pr\{r\}$	D_r^*	$Pr\{r\} \cdot D_r$
1, 2	0.0000		0.0000
3	0.0011	2.932	0.0032
4	0.0204	2.787	0.0569
5	0.1957	2.597	0.5082
Sum	0.2172		0.5683

$$D_{exp} = \frac{\sum_{r=1}^{n-1} (Pr\{r\} \cdot D_r)}{\sum_{r=1}^{n-1} Pr\{r\}} = \frac{0.5683}{0.2172} = 2.616$$

* Calculated by (4).

Consider the period $t = 13.20$. The probabilities of obtaining 5 or 4 sterile tubes are appreciable; of obtaining 3, 2, 1, or 0 are vanishingly small. The probability of obtaining 6 sterile tubes is large, and if this happens a value for D is not calculated. The expected value for D, if one is calculated, is obtained as a weighted average as is illustrated in table 1. Values obtained in this way are plotted to give the expected value of D as a function of the time of heating (figure 2). For short periods of heating D_{exp} is less than the theoretical D, D_{th}; for long periods D_{exp} is greater than D_{th}. Clearly this arises from rejecting observations in which $r = 0$ or $r = n$. For short periods the rejected observations are mostly $r = 0$; for long periods, mostly $r = n$.

Because of the asymmetry of the curves of figure 1, the 50 per cent sterility time ($P = 0.50$; $t = 10 - \log_e(-\log_e 0.5) = 10.367$) does not give an expected D that is equal to D_{th}, 2.3026; instead $D_{exp} = 2.298$ by the method of table 1, or 0.2 per cent lower than 2.3026. By successive approximations, the time giving $D_{exp} = 2.3026$ was found to be 10.610 ($P = 0.581$) for $n = 6$. The further t is from this time the greater is the departure of the expected D from the D_{th}. For $n = 6$ the rate of change of D_{exp} with time approaches 0.2 per minute, or 8.7 per cent of the theoretical value, and is approximately 0.12 (or 5.2 per cent) per minute

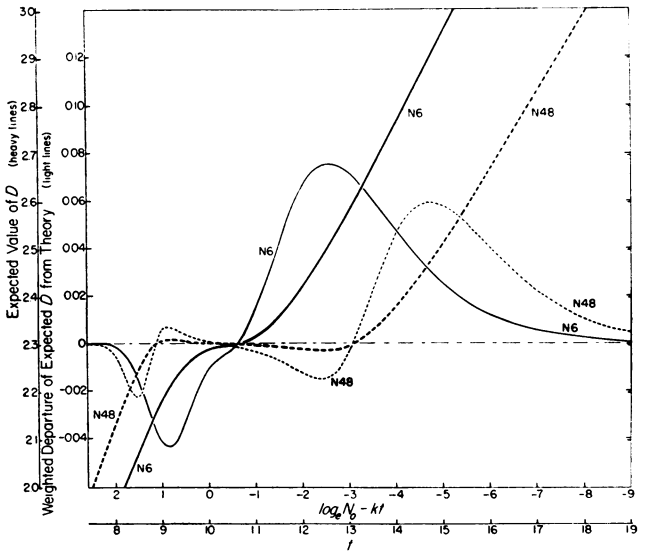

FIG. 2. Expected value of D for $n = 6$ and 48 when calculated by the "unweighted average" method (heavy lines). Weighted departure of the expected value of D from the theoretical $D = 2.3026$ (light lines).

for a range that embraces 95 per cent of the D values likely to be calculated. (For larger n the rate of change would be smaller.) This is in remarkable agreement with the values quoted by Reynolds and Lichtenstein for $n = 5$ to 12, and thus it appears entirely adequate to account for the effect that they noted with dilution counts. The bias illustrated would not appear to account for changes in D value with time when the data are plate counts, unless dilutions are so arranged as to give appreciable probabilities of sterile plates.

$D_{\exp}$ is also shown for $n = 48$ in figure 2. Nearly correct values for D are to be expected over a wider time range for $n = 48$ than for $n = 6$ but here also the values calculated for the shortest and longest times are strongly biased.

The unweighted average method with equal time intervals is biased to give somewhat too high values for D. The expected value for the unweighted average D is obtained as a weighted average of the expected values for individual time periods; that is, by

$$D_{unw,\ \exp} = \frac{\sum_{t=0}^{\infty} (D_{\exp} \cdot Pr\{r \neq 0, n\}_t}{\sum_{t=0}^{\infty} (Pr\{r \neq 0, n\})_t} \qquad (13)$$

where $Pr\{r \neq 0, n\}$ gives the probability that some but not all of the samples will be sterilized at a given time period (and thus that a value of D will be calculated for that period). The value of (13) will fluctuate with the size of the time intervals and the way they happen to fall on the range of partial sterilization, but as the size of the time intervals decreases (13) will approach

$$\frac{\int_{t=0}^{\infty} D_{\exp} \cdot Pr\{r \neq 0, n\} \cdot dt}{\int_{t=0}^{\infty} Pr\{r \neq 0, n\} \cdot dt} = \frac{\int_{t=0}^{\infty} (D_{\exp} - D_{th}) \cdot Pr\{r \neq 0, n\} \cdot dt}{\int_{t=0}^{\infty} Pr\{r \neq 0, n\} \cdot dt} + D_{th} \qquad (14)$$

The right-hand side of identity (14) contains an expression for the weighted departure from theory, and this may be evaluated graphically more accurately than the expression on the left-hand side of (14).

The top integral, the weighted departure, is given in figure 2 for $n = 6$; the bottom integral is given in figure 1. Planimetric evaluation showed that the overall bias is about 2.7 per cent too high. If the range of partial sterilization is curtailed, the bias is greater; thus, evaluation for $t \geqq 10.61$ gave a bias of 6.1 per cent and more extreme curtailment would give a greater bias. The weighted departure for $n = 48$ is also shown in figure 2; its form is more complex than for $n = 6$ but here also the overall bias is on the high side.

Although this bias and other defects of the unweighted average method (such as the lack of an estimate of precision) may be negligible in many applications, it would seem preferable to use a theoretically superior method of calculation if the labor were not increased unduly. Schmidt (1954) suggested the use of a procedure described by Reed (1936) that approximates graphically the time corresponding to 50 per cent sterility (in a symmetrical tolerance distribution). It is superior to the unweighted average method in that it gives less undue weight to observations at the extremes of the partial response range. The writer has found no detailed analysis of this method, but a closely-related method (Reed-Muench) has been discussed by Finney (1952; par. 20.8, 20.11, 20.12). Finney (Chap. 19, 20) has discussed the problem of estimates from quantal responses very thoroughly. From the standpoints of mathematical rigor and efficient experimental design he prefers a maximum likelihood method. Such calculations may be relatively tedious, as will be illustrated below; of alternative methods the Spearman-Kärber appears to be most applicable to data of the form usually collected.

THE SPEARMAN-KÄRBER METHOD

This method for the reduction of binomial data has been discussed critically by Finney (1952; paragraphs 20.6, 20.11, 20.12). It may be used to estimate the mean sterility time, t_m, of a sample and the variance of this estimate, V_{t_m}, by the formulae given in table 2. The estimates of D and k (or of confidence limits for D and

TABLE 2. *Formulae for the Spearman-Kärber method; estimates of the mean sterility time, t_m, and its variance,* V_{t_m}*

General case:

$$t_m = \sum_{i=1}^{u} \left(\frac{t_{i+1} + t_i}{2}\right)\left(\frac{r_{i+1}}{n_{i+1}} - \frac{r_i}{n_i}\right) \tag{15}$$

$$V_{t_m} = \sum_{i=1}^{u} \left(\frac{t_{i-1} - t_{i+1}}{2}\right)^2 \cdot \left(\frac{P_i(1 - P_i)}{n_i}\right) \cong \sum_{i=1}^{u} \left(\frac{t_{i-1} - t_{i+1}}{2}\right)^2 \cdot \left(\frac{r_i(n_i - r_i)}{n_i^2(n_i - 1)}\right) \tag{16}$$

Successive periods differ by equal time intervals, d:

$$t_m = t_u + \frac{d}{2} - d \sum_{i=1}^{u} \frac{r_i}{n_i} \tag{17}$$

$$V_{t_m} = d^2 \sum_{i=1}^{u} \left(\frac{P_i(1 - P_i)}{n_i}\right) \cong d^2 \sum_{i=1}^{u} \left(\frac{r_i(n_i - r_i)}{n_i^2(n_i - 1)}\right) \tag{18}$$

All periods involve an equal number of tests, n.

$$t_m = \frac{1}{n} \sum_{i=1}^{u} \left(\frac{t_{i+1} + t_i}{2}\right)(r_{i+1} - r_i) \tag{19}$$

$$V_{t_m} = \frac{1}{n} \sum_{i=1}^{u} \left(\frac{t_{i-1} - t_{i+1}}{2}\right)^2 (P_i(1 - P_i)) \cong \frac{1}{n^2(n-1)} \sum_{i=1}^{u} \left(\frac{t_{i-1} - t_{i+1}}{2}\right)^2 (r_i(n - r_i)) \tag{20}$$

Both d and n are constant:

$$t_m = t_u + \frac{d}{2} - \frac{d}{n} \sum_{i=1}^{u} r_i \tag{21}$$

$$V_{t_m} = \frac{d^2}{n} \sum_{i=1}^{u} (P_i(1 - P_i)) = \frac{d^2}{n^2(n-1)} \sum_{i=1}^{u} (r_i(n - r_i)) \tag{22}$$

Summations are carried out in theory over all time periods; in practice, terms beyond a certain time, t_u, have no effect. In formulae that do not involve P_i, t_u may be any period beyond all those that give one or more viable samples. P_i is the theoretical probability of a sample being sterile at t_i, and $p_i = \frac{r_i}{n_i}$ is an estimate of P_i. Better estimates of P_i, for use with the exact equations for V_{t_m}, may be calculated by (28) by means of a table of loglogs (Finney, 1952, Appendix Table XVI—see the next section) and k estimated by equation (25).

The 95 per cent confidence limits for t_m are taken as

$$t_m \pm 1.96(V_{t_m})^{\frac{1}{2}} \tag{23}$$

from the normal probability distribution as an approximation for the distribution of t_m.

* The variance is the square of the standard deviation. The general formula is not given by Finney but it may be obtained from (15) by a conventional procedure described by Wilson (1952, p. 272).

k) are obtained by substituting t_m (or the confidence limits for t_m) in

$$D = \frac{2.3026\, t_m}{\log_e N_0 + 0.61} = \frac{t_m}{\log_{10} N_0 + 0.265} \tag{24}$$

and

$$k = \frac{\log_e N_0 + 0.61}{t_m} \tag{25}$$

An example of the calculation is given in table 3.

TABLE 3. *Estimation of survival rate constant k (or decimal reduction time D) by the Spearman-Kärber method*

Spores of *Clostridium* sp. PA 3679 in pea puree, $N_0 = 2.64 \times 10^4$, 120.1C., $d = 0.5$ minute throughout (data of O'Brien *et al.*, 1956)

t_i	r_i/n_i	$r_i/n_i - r_i)/n_i^2(n_i - 1)$	$Y = \log_e \frac{N_0}{kt}$ (by (29))	$P = e^{-eY}$ (by (28))
5.5	0/5	0	1.93	.001
6.0	1/6	5/180	1.18	.039
6.5	1/6	5/180	0.43	.215
7.0	2/6	8/180	−0.32	.483
7.5	5/6	5/180	−1.07	.710
8.0	5/6	5/180	−1.82	.850
8.5	4/5	4/100	−2.57	.926
9.0	5/5	0	−3.32	.964
9.5	5/5	0	−4.07	.983
10.0	5/5	0	−4.82	.992
10.5	6/6	0	−5.57	.996
11.0	5/5	0	−6.32	.998
	$\sum_{t_i=5.5}^{8.5}$	$\sum_{t_i=5.5}^{8.5}$		$d^2 \sum_{t_i=0}^{\infty} \frac{P(1-P)}{n}$
	$= \frac{14}{6} + \frac{4}{5}$	$= \frac{28}{180} + \frac{4}{100}$		$= \frac{0.1309}{5\times4} + \frac{0.7933}{6\times4}$
	$= 3.133$	$= 0.1955$		$= 0.0396$

By (17)

$$t_m = t_u + \frac{d}{2} - d \sum r_i/n_i$$
$$= 8.5 + 0.25 - 0.5 \times 3.133$$
$$= 7.183$$

And by (18)

$$V = d^2 \sum (r_i(n_i - r_i)/n_i^2(n_i - 1))$$
$$= 0.25 \times 0.1955$$
$$= 0.0489$$

Then

$$1.96s = 1.96(V)^{\frac{1}{2}} = 1.96 \times .221$$
$$= 0.434$$

and

$$t_m = 7.183 \pm 0.434$$

By (24)

$$D = \frac{2.303\ (7.183 \pm 0.434)}{10.181 + 0.61}$$
$$= 1.53 \text{ with confidence limits } 1.44 \text{ to } 1.63$$

By (2) or (25)

$$k = 1.50 \text{ (95\% CL } 1.42 \text{ to } 1.60).$$

In the preceding calculations t_u was taken as 8.5; a higher value would have given the identical results.

Alternatively V may be calculated by (18) with P_i estimated by (28); this gives $V = 0.0396$ and $k = 1.42$ to 1.59.

Formulae (24) and (25) are taken from (4) and (2) with $\log_e N = \log_e(-\log_e P_m) = 0.61$ corresponding to $P_m = 0.58$. This value for P_m was obtained by estimating the bias of the Spearman-Kärber method for

TABLE 4. *Maximum likelihood estimation of k after loglog transformation of response (data as in table 3)*

t_i	r_i/n_i	Y_i	η_i	y_i	w_i
5.5	0/5	1.9	−0.15	2.05	0.047 (*i.e.*, $\frac{5}{6} \times 0.056$)
6.0	1/6	1.2	1.09	0.11	0.413
6.5	1/6	0.4	−0.17	0.57	0.646
7.0	2/6	−0.3	−0.41	0.11	0.500
7.5	5/6	−1.1	0.49	−1.59	0.281
8.0	5/6	−1.8	−0.10	−1.70	0.152
8.5	4/5	−2.6	−1.86	−0.74	0.060 (*i.e.*, $\frac{5}{6} \times 0.072$)
9.0	5/5	−3.3	1.02	−4.32	0.030 (*i.e.*, $\frac{5}{6} \times 0.036$)
9.5	5/5	−4.1	1.01	−5.11	0.014 (*i.e.*, $\frac{5}{6} \times 0.016$)
10.0	5/5	−4.8	1.00	−5.80	0.007 (*i.e.*, $\frac{5}{6} \times 0.008$)
10.5	6/6	−5.6	1.00	−6.60	0.004
11.0	5/5	−6.3	1.00	−7.30	0.002 (*i.e.*, $\frac{5}{6} \times 0.002$)
$\sum w_i t_i = 14.806$				$\sum w_i y_i = -0.467$	$\sum w_i = 2.156$

By (29), $Y_i = \log_e N_0 - kt_i = 10.18 - 1.50\, t_i$. One decimal place in Y_i ordinarily suffices.

The working deviate η_i is obtained from Y_i and $P_i = \frac{r_i}{n_i}$ by interpolation in Finney's Appendix Table XVII by means of formulae 21.60, 21.61, and 21.62, p. 577. The working loglog y_i is obtained by

$$y_i = Y_i - \eta_i \tag{30}$$

The weighting coefficient w_i is obtained from Y_i by interpolation in Finney's Appendix Table XVII. When n_i is not constant it is convenient to adjust w_i to a common basis by multiplying w_i by $\frac{n_i}{n}$, as has been done in the table with $n = 6$, and using n in the equation for variance (34).
By (31),

$$k = \frac{(\log_e N_0) \sum w_i - \sum w_i y_i}{\sum w_i t_i}$$

$$= \frac{10.18 \times 2.156 + 0.467}{14.806} = 1.514$$

This estimate may be assumed not to differ sufficiently from the Spearman-Kärber estimate to warrant another cycle of calculation. If it were thought to differ sufficiently another cycle of calculations starting with $Y_i = 10.18 - 1.514\, t_i$ could be made, and in any event it would serve as a check on the previous computation.

Conformity of the data to the binomial distribution should be checked next. The number in each group, n_i, is too small to make the chi square test, (32) or (33), appropriate. A test by means of confidence limits for Y is illustrated in the first paragraph of the last section of this paper. None of the confidence limits for k estimated in this manner for the individual time periods are inconsistent with the loglog estimate of k; thus, there is no evidence of nonconformance to the binomial distribution. The test can be made very rapidly inasmuch as it need be applied only to the most suspected periods; for example, $t = 6.0$ and 8.5 in this table.

TABLE 4.—*Continued*

Assuming N_0 is known exactly, the theoretical variance for k, by (34), is

$$V_k = \frac{\sum w_i}{n(\sum w_i t_i)^2} = \frac{2.156}{6(14.806)^2} = 0.00164.$$

The 95% confidence limits are given by

$$k \pm 1.96\, V_k^{\frac{1}{2}} = 1.52 \pm 0.08 = 1.44 \text{ to } 1.60.$$

The significance of this estimate is discussed in the text, and incorporation of the variance of $\log_e N_0$ is illustrated in the first footnote to table 6.

the limiting case of infinitesimally small equally spaced time periods and the data of figure 1. Equation (17) then becomes

$$t_m = t_u - \int_{t=0}^{\infty} P\, dt. \tag{26}$$

Planimetry of the integral, shown in figure 1, gave $t_m = 10.61$ and thus $P_m = 0.58$ by (11).

It is preferable that successive time periods be fairly close together, that the number of samples at each period and the intervals between successive periods be constant, and that the range of partial sterilization be bracketed completely. The requirement for a considerable number of test periods throughout the partial sterilization range implies that many tests will be made at times that do not yield the most information. If the response range can be predicted well, the maximum likelihood (loglog) method described below may allow enough saving in experimentation to compensate for the more laborious computations.

MAXIMUM LIKELIHOOD ESTIMATION OF k AFTER LOGLOG TRANSFORMATION OF RESPONSE (THE LOGLOG METHOD)

Finney has discussed the general problem of computing biological assays by estimating the parameters of "tolerance distributions," whether real or assumed because of the usefulness of the mathematical form, through transformations to provide a linear dependence of a function of the response on a function of the dose. Thus

$$Y = \alpha + \beta x \tag{27}$$

where Y is the transformed response (the "response metameter"), x is the transformed dose, β is the parameter of the tolerance distribution that is determined by the units of the dose scale, and α is the parameter that is determined by the position of the partial response range on the dose scale.

The tolerance distribution of samples of microorganisms that follow exponential survival when exposed to heat is given by (11) which may be written as

$$\log_e (-\log_e P) = \log_e N_0 - kt \tag{28}$$

which is identical in mathematical form to (27). The first term, which will be denoted as Y, is a response metameter named the loglog. It was proposed by Mather (1949) who gave the solution of (27) for both α and β. Finney (paragraph 21.5) has illustrated the solution of (27) for a dilution count; in this case β is given by $\log_e$ of the known dilution ratio, and $\alpha = \log_e N_0$ is estimated. In its simplest form, the estimation of decimal reduction times involves the complementary problem of estimating β (that is, $-k$) where $\alpha = \log_e N_0$ is known. A transformation of dose is not necessary; that is, $x = t$. The computations are somewhat more involved than those involved in the dilution count, and are illustrated in table 4 with the same example that was used to illustrate the Spearman-Kärber method (table 3).

The method consists of successive cycles of computation that yield estimates of k that approach the theoretical maximum likelihood estimate asymptotically. A good guess for the initial estimate may eliminate one or more cycles of the calculation. Thus it may be desirable to make an initial estimate by the Spearman-Kärber method. This estimate of k, 1.50, is used for the first cycle of computation shown in table 4. In the first column is recorded the time of heating; in the second column the observed proportion of sterile samples, $p_i = r_i/n_i$; in the third column the initial estimates of Y which are calculated by

$$Y = \log_e N_0 - kt \tag{29}$$

Other methods of obtaining the initial estimates of Y are discussed by Finney. In columns 4, 5, and 6 are given the "working deviate" η, the "working loglog" y_i , and the "weighting coefficient" w_i .

These columns provide the material for the first cycle of computation. In the formulae now to be given n will be assumed variable inasmuch as the simplified formulae for n constant will be obvious. The following summations (over all test periods) are required: $\sum n_i w_i$, $\sum n_i w_i t_i$, $\sum n_i w_i y_i$. The sign of y_i must be retained. The revised estimate of k is given by

$$k = \frac{\log_e N_0 \cdot \sum n_i w_i - \sum n_i w_i y_i}{\sum n_i w_i t_i} \tag{31}$$

This value of k is used to compute new values of Y_i by (29). A revised estimate of k is obtained by a new cycle of computation, and the process is repeated until the change in successive estimates of k becomes negligible.

When a good estimate of k has been obtained the homogeneity of the data may be tested by

$$\chi^2_{\nu-1} = \sum \frac{n_i(r_i - E(r_i))^2}{E(r_i)(n_i - E(r_i))} \tag{32}$$

where the summation is carried over the ν periods tested and $E(r_i)$ is the expected value of r_i , $n_i P_i$. An approximate expression for (32) is

$$\chi^2_{\nu-1} = \sum n_i w_i y_i^2 - \frac{(\sum n_i w_i y_i)^2}{\sum n_i w_i} + k\left[\sum n_i w_i y_i t_i - \frac{(\sum n_i w_i y_i)(\sum n_i w_i t_i)}{\sum n_i w_i}\right] \tag{33}$$

The probability of $\chi^2_{\nu-1}$ is determined from a chi square table for $\nu - 1$ degrees of freedom; if the value was improbable the individual terms of (32) should be inspected to see if the contributions from periods with $E(r_i)$ less than 5 are unduly large. If so, small classes may be combined (with a reduction in degrees of freedom) or exact binomial confidence limits may be calculated as described in the final section. With n as small as in table 4 the latter procedure is preferable and is illustrated in table 7.

The test with confidence limits for individual periods would not be expected to disclose small systematic deviations from the exponential survival rule unless n were large. A qualitative judgment might be made by plotting a large number of confidence limits, and inspecting for systematic trends. If the discrepancies were non-systematic but abnormally frequent (see the last section of this paper), a reinvestigation of the experimental procedure would appear to be in order.

Confidence limits of k for valid data may be obtained from the expected variance of k, V_k , for the distribution assumed and the tests made by

$$V_k = \sum n_i w_i / (\sum n_i w_i t_i)^2. \tag{34}$$

Inasmuch as this is not an empirical estimate of variance, its use should be restricted to data that meet the validity test. Confidence limits are calculated by the normal distribution, or more conservatively by the t distribution with degrees of freedom equal to the total number of samples in the partial response range diminished by the corresponding number of periods tested.

It has been assumed that N_0 is known exactly, inasmuch as it ordinarily is determined independently and thus theoretically to any desired accuracy. A more common case is that N_0 is indeed constant (pipetting or related errors are negligible) but is not known with high precision. This is true of the data of tables 3, 4, and 6. The confidence intervals shown are suitable for comparing treatments (presence or absence of subtilin; relative initial number of spores), but they fail to include the contribution of variance of the estimate of $\log_e N_0$, $V_{\log_e N_0}$. This is accomplished by the equation

$$V_k = \left(\frac{\sum n_i w_i}{\sum n_i w_i t_i}\right)^2 \left[V_{\log_e N_0} + \frac{1}{\sum n_i w_i}\right] = \left(\frac{\sum n_i w_i}{\sum n_i w_i t_i}\right)^2 V_{\log_e N_0} + \frac{\sum n_i w_i}{(\sum n_i w_i t_i)^2}. \tag{35}$$

If both k and $\log_e N_0$ must be estimated from the binomial data (see Mather, 1949) the precision of the estimates will be greatly reduced.

The loglog method of estimation of k with a single time period gives a result identical with that given by the unweighted average method. Thus k is obtained directly from (31) by setting

$$y = Y = \text{loglog } p = \log_e \log_e (n/r). \qquad (36)$$

(Finney's Appendix Table XVI and National Bureau of Standards 1953, table 2, give this function.) Then

$$k = \frac{\log_e N_0 - y}{t} = \frac{\log_e N_0 - \log_e \log_e(n/r)}{t} \qquad (37)$$

which may be obtained by combining equations (4) and (2) of the unweighted average method. The variance for the loglog estimate, which would be equally appropriate for the single period of the unweighted average method, is

$$V_k = \frac{1}{nwt^2}. \qquad (38)$$

It may be seen (by Finney's Appendix Table XVII or in the figure given by Mather) that (38) will be minimal for p equal to 0.20 approximately; that is, the estimate will be most precise for this proportion of sterile samples. It is the essential distinction between the two methods that varying reliability of the data is taken into account in the loglog calculation but not in the unweighted average calculation.

Thus it becomes clear why use of the loglog method can lead to far more efficient experimentation if the outcome can be predicted fairly well. Suppose that all of the tubes for the data of table 3 (and 4) had been utilized at the period $t = 6.5$. The variance of the estimate of k would have been approximately

$$\frac{\sum n_i w_i}{\sum nw} = \frac{6 \times 2.16}{66 \times 0.65} = 0.30$$

as great; that is, the confidence interval would have been less than six-tenths as wide. Put another way, the omission of periods $t = 8.5$ and longer would not have reduced the accuracy of the result appreciably. The advantage of reducing the number of dose levels in speeding the computations will also be noted.

Data that show a sharp cutoff have not been reducible satisfactorily by the unweighted average method but they present no problem to the loglog method. Consider the arbitrary example shown in table 5. The data for cases I and II have been chosen as reasonably coming from the same population with the respective guesses of appropriate time periods. Not only are the results equally, readily, calculable but their reliabilities are virtually identical.

Similarly, data from curtailed response ranges offer no difficulties for the loglog method. Such data give biases in the unweighted average method, and their effect on the Spearman-Kärber method is not known.

A more complicated situation arises where negative exponential survival is not observed; that is, where equation (1) does not hold. A common type of departure, illustrated by a sagging curve rather than a linear relationship of $\log_e \frac{N}{N_0}$ *vs.* time, is given by a population mixed with respect to resistance (k (or D) variable). This phenomenon has been discussed by Rahn (1945, p. 19). It is demonstrated in a figure given by O'Brien *et al.* (1956) for heat killing of *Clostridium sp.* PA 3679 with various initial numbers of spores. Part of the original data have been treated by the various methods discussed in this paper with the results shown in table 6.

Where exponential survival holds (the "controls" of table 6) the agreement of the three methods is very close. Where exponential survival does not hold ("subtilin present" of table 6) the agreement is very poor for the lowest initial spore number. The confidence limits calculated in the various ways shown also agree well except with subtilin and the lowest initial spore number. Exact confidence limits for k calculated from the binomial probabilities (as outlined in the next section) for individual time periods agreed within the 5 per cent level with the confidence limits shown in table 6 in all cases.

The overlapping of the confidence ranges of k for various N_0 in the controls and the failure to overlap in the presence of subtilin demonstrates a departure from exponential survival in the latter case but fails to demonstrate a departure in the former case.

It does not appear to be possible to make a sensitive test for conformance to exponential survival by any mathematical device when only one inoculum level

TABLE 5. *Loglog transformation for data with a sharp cutoff (arbitrary example; $\log_e N_0 = 5$; $n = 6$)*

t	r	
	Case I	Case II
5	0	
6		0
7	0	
8		0
9	3	
10		6
11	6	
12		6
13	6	
14		6
15	6	

Case I: $k = 0.599 \pm 0.080$
Case II: $k - 0.594 \pm 0.079$
Combined data: $k = 0.\ 0.597 \pm 0.057$
(95% confidence intervals)

TABLE 6. *Comparison of methods for calculating survival rate constants (extension of the data of tables 3 and 4)*

Initial Number of Spores (N_0)*	Original Data†	Estimations of k with 95% Confidence Intervals		
		Unweighted average‡	Spearman-Kärber	Loglog
Controls				
2.64×10^1	0.5 (by 0.5) to 6	1.24	1.33	1.28
	$\frac{0, 0, 0, 0, 2, 5, 4, 6, 6, 5, 6, 6}{6}$		(1.18–1.53)§	(1.09–1.47)
			(1.17–1.55)¶	
2.64×10^2	3.5 (by 0.5) to 9	1.46	1.43	1.43
	$\frac{0, 0, 1, 0, 0}{6}\ \frac{3}{5}\ \frac{6, 5, 6, 6}{6}\ \frac{5, 5}{5}$		(1.35–1.51)§	(1.34–1.52)
			(1.34–1.53)¶	
2.64×10^4	5.5 (by 0.5) to 11	1.49	1.50	1.52
	$\frac{0}{5}\ \frac{1, 1, 2, 5, 5}{6}\ \frac{4, 5, 5, 5}{5}\ \frac{6}{6}\ \frac{5}{5}$		(1.42–1.60)§	(1.44–1.60)
			(1.42–1.59)¶	
Subtilin present				
2.64×10^1	0.4 (by 0.2) to 2.6	5.31	5.82	6.04
	$\frac{1, 4, 3, 6, 5, 6, 6, 6, 6, 6, 6, 6}{6}$		4.75–7.54)§	(4.84–7.24)
			(4.92–7.14)¶	
2.64×10^2	1.8 (by 0.2) to 4	3.12	3.09	3.18
	$\frac{0}{5}\ \frac{1, 1, 0, 3}{6}\ \frac{3}{5}\ \frac{4, 4}{6}\ \frac{5, 5}{5}\ \frac{6}{6}\ \frac{5}{5}$		(2.88–3.33)§	(2.98–3.38)
			(2.92–3.29)¶	
2.64×10^4	2.8 (by 0.2) to 5	2.97	2.99	3.05
	$\frac{1}{5}\ \frac{0, 2}{6}\ \frac{1}{4}\ \frac{3, 3, 5, 6, 5, 6, 6, 6}{6}$		(2.83–3.16)§	(2.90–3.20)
			(2.86–3.13)¶	

* For comparison of estimation methods and experimental treatments in this table, N_0 has been assumed known exactly from independent determinations. If absolute values for the confidence intervals were desired it would be necessary to introduce the variance of $\log_e N_0$ by (35). With low N_0 the Poisson variance of N_0 (which is equal to N_0) for equal volumes of the sample becomes important for relative values of confidence intervals such as have been calculated here. Inserting the Poisson component of variance of $\log_e N_0$, $\frac{1}{N_0}$, in (35) gives

$$V_k = \left(\frac{\sum n_i w_i}{\sum n_i w_i l_i}\right)^2 \left(\frac{1}{N_0} + \frac{1}{\sum n_i w_i}\right) \tag{39}$$

For $N = 26.4$ in the control group

$$V_k = \left(\frac{15.2}{40.5}\right)^2 (0.038 + 0.066)$$

which gives a confidence interval of ± 0.24 instead of the ± 0.19 shown in the table. The effect is much less marked in the other cases.

The accuracy of the approximation involved in equation (9) may be considered here. Retention of the second term of the approximation; that is,

$$\log_e P = -N_0 \left(e^{-kt} + \frac{e^{-2kt}}{2}\right)$$

and calculation of the Spearman-Kärber estimates of k with $N_0 = 26.4$ gave values that differed from those shown in the table by less than 0.5%.

TABLE 6.—*Continued*

† Note that the third code given below expresses the data given in table 3.

‡ By $k = \frac{2.3026}{\hat{D}_{unw}}$ where $\hat{D}_{unw}$ is computed by (4) and (5). More simply, by

$$k = \frac{j}{\sum_{i=1}^{i} \left(\frac{t_i}{\log_e N_0 - \log_e \log_e \frac{n_i}{r_i}} \right)} \tag{40}$$

§ Calculated as described in table 3, using r_i and n_i in (18).

¶ Calculated as described in table 3, using (18) with P_i estimated by (28).

(N_0) is available. By far the best procedure is the use of widely-separated values of $\log_e N_0$.

CONFIDENCE LIMITS FOR BINOMIALLY DISTRIBUTED DATA

It is sometimes necessary to check in detail the basic assumption of a binomial distribution as well as the conformance to an assumed relationship of P to t. For tests of exponential survival it is convenient to have the confidence limits of $Y = \log_e(-\log_e P)$ rather than the confidence limits of P. These are given for $n \leq 16$, $\alpha = 0.05$ and 0.01, in table 7. An example of their use follows: From the data of table 6, take the case of $N_0 = 2.64 \times 10^3$, subtilin present, $t = 2$ minutes, $r/n = \frac{1}{6}$ (that is, one of six samples was sterile). Is this result consistent with the estimations of k shown in table 6? The confidence limits for Y, $\alpha = 0.05$, are 1.56, −0.61. Rewriting equation (29)

$$k_j = \frac{\log_e N_0 - Y_j}{t} \tag{46}$$

where j indicates one of the confidence limits. Then

$$k_1 = \frac{7.88 - 1.56}{2} = 3.16$$

$$k_2 = \frac{7.88 + 0.61}{2} = 4.24$$

Similarly for $\alpha = 0.01$

$$k_1 = 3.02, \; k_2 = 4.46$$

By comparison with the confidence intervals of table 6 it is seen that an estimation from this portion of the data, representing a relatively short time of heating, does not depart from the estimations from all the data with significance even at 5 per cent.

Confidence intervals have been calculated for the data of Pflug and Esselen (1954), for which $n = 48$, because the bias in the unweighted average method of calculation that they used is inadequate to account for the deviations that they found from predictions based on exponentially decreasing survival. The nature of the discrepancies become more clear if the probability that an individual spore survives is plotted on a logarithmic scale against time of heating on a linear scale. This probability, Π, is given by the ratio of the final to the original number of spores; by (1) and (9)

$$\begin{aligned} \log_e \Pi &= \log_e (N/N_0) \\ &= -kt = \log_e(-\log_e P) - \log_e N_0 \\ &= Y - \log_e N_0 . \end{aligned} \tag{47}$$

By substituting the confidence limits for Y in (47) the 99 per cent confidence intervals for $\log_e \Pi$ shown as vertical bars in figure 3 were obtained. Those that correspond to $r_i = 0$ or n_i are unbounded on top or bottom. The diagonal lines through $\log_e \Pi = 0$ give predicted values of $\log_e \Pi$ for loglog estimates of k.

As Pflug and Esselen point out, the data deviate markedly from expectation for exponential survival. It is more pertinent, however, that the data also deviate markedly from expectation for a binomial distribution. Three discrepancies will be detailed. One may first consider the confidence intervals marked A corresponding to the times 0.0562 and 0.0599 and $N_0 = 120$. No reasonable course for $\log_e \Pi$ could pass near both of these intervals. On the reasonable assumption that $\log_e \Pi = -90.65t$ in this local region the probability of obtaining a value of r as low as that observed (0) at $t = 0.0562$ and a value as high as that observed (45) at $t - 0.0569$ would be infinitesimal, much less than 10^{-14}. As a matter of fact, no reasonable course for $\log_e \Pi$ can be drawn through either one of these confidence limits and the others for $N_0 = 120$.

Even more marked discrepancies are apparent between sets of data for the various initial numbers. Attention is directed to the regions B and C that are bounded roughly by dotted lines. Region B has at least 10 confidence intervals that are inconsistent with any reasonable single-valued function for $\log_e \Pi$; region C has at least 7. If it be assumed that the initial concentration of spores was not known, marked changes in the dilution ratios (that is, to less than $\frac{1}{15}$ instead of $\frac{1}{85}$ between $N_0 = 120$ and $N_0 = 10^4$) would have to be assumed to reconcile region B.

It perhaps is idle to speculate further as to the nature of the extraneous factors that have given these anomalous data; they point up the need for statistical control even with an experimental setup as apparently

TABLE 7. *Confidence intervals* for* $Y = \log_e(-\log_e P)$, *based on exact binomial probabilities for* $n = 1$ *to* 16

Boldface gives 99% confidence level ($\alpha = 0.01$). Regular type gives 95% confidence level ($\alpha = 0.05$).

$n = 1$	$n = 2$	$n = 3$	$n = 4$	$n = 5$	$n = 6$	$n = 7$	$n = 8$	$n = 9$	$n = 10$	$n = 11$	$= 12$	$n = 13$	$n = 14$	$n = 15$	$n = 16$	r
∞, −4.60 ∞, −2.97	**∞, −2.25** ∞, −1.37	**∞, −1.42** ∞, −0.78	**∞, −0.97** ∞, −0.45	**∞, −0.68** ∞, −0.43	**∞, −0.47** ∞, −0.25	**∞, −0.46** ∞, −0.11	**∞, −0.32** ∞, 0.00	**∞, −0.21** ∞, 0.09	**∞, −0.12** ∞, 0.16	**∞, −0.04** ∞, 0.23	**∞, 0.03** ∞, 0.28	**∞, 0.09** ∞, 0.34	**∞, 0.14** ∞, 0.38	**∞, 0.19** ∞, 0.42	**∞, 0.24** ∞, 0.46	0
1.53, −∞ 1.10, −∞	**1.67, −5.29** 1.30, −3.66	**1.74, −2.80** 1.41, −1.93	**1.79, −1.88** 1.47, −1.25	**1.83, −1.38** 1.52, −0.87	**1.86, −1.05** 1.56, −0.61	**1.88, −0.82** 1.59, −0.60	**1.90, −0.64** 1.62, −0.44	**1.92, −0.62** 1.64, −0.32	**1.93, −0.50** 1.66, −0.21	**1.95, −0.39** 1.68, −0.12	**1.96, −0.30** 1.70, −0.05	**1.97, −0.22** 1.71, 0.02	**1.98, −0.15** 1.72, 0.08	**1.99, −0.09** 1.74, 0.13	**2.00, −0.04** 1.75 0.18	1
$r = 2$	**0.83, −∞** 0.40, −∞	**1.04, −5.70** 0.69, −4.07	**1.15, −3.15** 0.84, −2.28	**1.23, −2.19** 0.94, −1.56	**1.29, −1.66** 1.02, −1.15	**1.33, −1.31** 1.08, −0.87	**1.37, −1.06** 1.12, −0.85	**1.40, −0.86** 1.16, −0.67	**1.43, −0.84** 1.19, −0.53	**1.45, −0.70** 1.22, −0.42	**1.47, −0.59** 1.25, −0.32	**1.49, −0.49** 1.27, −0.24	**1.51, −0.40** 1.29, −0.16	**1.52, −0.33** 1.31, −0.10	**1.54, −0.26** 1.33 −0.04	2
	$r = 3$	**0.43, −∞** 0.00 −∞	**0.67, −5.99** 0.33, −4.36	**0.81, −3.40** 0.51, −2.53	**0.90, −2.42** 0.63, −1.79	**0.97, −1.87** 0.72, −1.37	**1.03, −1.51** 0.79 −1.07	**1.08, −1.25** 0.84, −1.03	**1.11, −1.04** 0.89 −0.85	**1.15, −0.88** 0.93 −0.70	**1.18, −0.86** 0.97 −0.58	**1.20, −0.74** 1.00 −0.48	**1.23, −0.64** 1.03, −0.39	**1.25, −0.55** 1.05, −0.31	**1.27, −0.47** 1.08 −0.24	3
		$r = 4$	**0.14, −∞** −0.29, −∞	**0.41, −6.21** 0.07, −4.58	**0.56, −3.61** 0.27, −2.73	**0.67, −2.61** 0.40, −1.98	**0.75, −2.05** 0.50, −1.54	**0.81, −1.67** 0.58, −1.24	**0.86, −1.40** 0.64, −1.02	**0.91, −1.19** 0.69, −1.00	**0.95, −1.02** 0.74, −0.85	**0.98, −1.00** 0.78, −0.72	**1.01, −0.87** 0.82, −0.61	**1.04, −0.76** 0.85, −0.52	**1.06, −0.67** 0.88, −0.44	4
			$r = 5$	**−0.08, −∞** −0.30, −∞	**0.20, −6.39** −0.14, −4.76	**0.37, −3.78** 0.07, −2.90	**0.48, −2.77** 0.22, −2.14	**0.57, −2.20** 0.32, −1.69	**0.64, −1.81** 0.41, −1.38	**0.70, −1.53** 0.48, −1.15	**0.75, −1.32** 0.54, −1.13	**0.79, −1.14** 0.59 −0.97	**0.83, −1.11** 0.63, −0.84	**0.86, −0.98** 0.67, −0.73	**0.89, −0.87** 0.71, −0.63	5
				$r = 6$	**−0.26, −∞** −0.49, −∞	**0.03, −6.55** −0.15, −4.92	**0.20, −3.92** 0.05, −3.05	**0.33, −2.90** 0.19, −2.28	**0.42, −2.32** 0.18, −1.82	**0.49, −1.94** 0.27, −1.50	**0.56, −1.65** 0.34, −1.27	**0.61, −1.43** 0.40, −1.24	**0.66, −1.25** 0.46, −1.08	**0.70, −1.22** 0.50, −0.94	**0.73, −1.08** 0.55, −0.83	6
					$r = 7$	**−0.28, −∞** −0.64, −∞	**−0.11, −6.68** −0.29, −5.05	**0.07, −4.05** −0.09, −3.17	**0.19, −3.02** 0.06, −2.39	**0.29, −2.44** 0.16, −1.93	**0.37, −2.04** 0.25, −1.61	**0.44, −1.75** 0.32, −1.37	**0.49, −1.53** 0.38, −1.34	**0.54, −1.35** 0.44, −1.17	**0.58, −1.31** 0.48, −1.04	7
						$r = 8$	**−0.41, −∞** −0.77 −∞	**−0.13, −6.80** −0.42 −5.17	**0.04, −4.16** −0.21 −3.28	**0.08, −3.13** −0.06 −2.50	**0.18, −2.54** 0.05 −2.03	**0.26, −2.14** 0.14, −1.71	**0.33, −1.85** 0.22 −1.47	**0.39, −1.62** 0.28 −1.43	**0.44, −1.44** 0.34 −1.26	8
							$r = 9$	**−0.53, −∞** −0.89, −∞	**−0.24, −6.90** −0.53, −5.27	**−0.06, −4.26** −0.32, −3.38	**0.06, −3.22** −0.16, −2.60	**0.16, −2.63** −0.05, −2.12	**0.24, −2.23** 0.04, −1.80	**0.31, −1.93** 0.12, −1.55	**0.37, −1.70** 0.19, −1.51	9
								$r = 10$	**−0.64, −∞** −1.00, −∞	**−0.34, −7.00** −0.63, −5.37	**−0.16, −4.35** −0.41, −3.48	**−0.03, −3.31** −0.26, −2.68	**0.07, −2.72** −0.14, −2.21	**0.15, −2.31** −0.04, −1.88	**0.22, −2.01** 0.04, −1.63	10
									$r = 11$	**−0.73, −∞** −1.09, −∞	**−0.43, −7.09** −0.72, −5.46	**−0.25, −4.43** −0.50, −3.56	**−0.12, −3.39** −0.34, −2.76	**−0.01, −2.79** −0.22, −2.29	**0.07, −2.39** −0.12, −1.95	11
										$r = 12$	**−0.82, −∞** −1.18, −∞	**−0.52, −7.17** −0.81, −5.54	**−0.33, −4.51** −0.58, −3.64	**−0.20, −3.47** −0.42, −2.84	**−0.09, −2.87** −0.30, −2.36	12
											$r = 13$	**−0.90, −∞** −1.26, −∞	**−0.59, −7.24** −0.88, −5.61	**−0.41, −4.58** −0.66, −3.71	**−0.27, −3.54** −0.49, −2.91	13
												$r = 14$	**−0.97, −∞** −1.33, −∞	**−0.67, −7.31** −0.95, −5.68	**−0.48, −4.65** −0.73, −3.77	14
													$r = 15$	**−1.04, −∞** −1.40, −∞	**−0.73, −7.37** −1.02, −5.74	15
														$r = 16$	**−1.11, −∞** −1.47, −∞	16

* Confidence intervals for Y are obtained from a classification of the possible combinations of r and P as probable or improbable. It is assumed that P is fixed (but unknown) in (hypothetically) repeated trials, whereas the possible values of r will have various probabilities depending upon the unknown value of P. Then for low values of P only the larger values of r can be improbable; thus, P cannot be so low as to make $r = o$ improbable. Similarly for high values of P only the smaller values of r can be improbable. For small values of n most of a linear P scale gives one-sided confidence limits for r. Accordingly, lower and upper confidence limits for r (r_1 and r_2, respectively) are defined by the condition that both

$$Pr\{r \leqq r_1\} \leqq \frac{\alpha}{2} \quad \text{and} \quad Pr\{r \geqq r_2\} \leqq \frac{\alpha}{2} \tag{41}$$

if possible, but that if

$$Pr\{r = 0\} > \alpha \quad \text{then} \quad Pr\{r \geqq r_2\} \leqq \alpha \tag{42}$$

or if

$$Pr\{r = n\} > \alpha \quad \text{then} \quad Pr\{r \leqq r_1\} \leqq \alpha \tag{43}$$

Inasmuch as r/n gives an estimate of P, confidence limits for P depend on the confidence limits for r. For a particular value of r, r_x, let $P_{1,x}$ and $P_{2,x}$ be the lower and upper confidence limits of the estimate of P obtained from the empirical value r/n. These are found by the following procedure: Find the value of P, $P_{2\,0}$, for which $Pr\{r = o\} = \alpha$ and with this value of P find r_j such that

$$Pr\{r > r_j\} \leqq \frac{\alpha}{2} < Pr\{r \leqq r_j\}. \tag{44}$$

For each $r_i \leqq r_j$ find the value of P, $P_{1,j}$, such that $Pr\{r \geqq r_i\} = \alpha$, and for each $r_k > r_j$ find $P_{1,k}$ such that $Pr\{r \geqq r_k\} = \frac{\alpha}{2}$. Find $P_{2,x}$ in analogous fashion or by means of the complementary property

$$P_{1,x} = 1 - P_{2,n-x} \tag{45}$$

Bennett and Franklin (1954, p. 604) and Wilson (1952, p. 182) have discussed confidence limits of the binomial distribution and have given charts that aid in visualizing the relationships. Neither source used the conditions defined in (41) to (43) which are particularly appropriate for small n (as is usual in thermal death studies); thus, only with $n \geqq 6$ does $P = 0.5$ ($\alpha = 0.05$) permit both improbably high and improbably low values of r. I am indebted to T. A. Jeeves for suggesting these conditions.

Values of $P_{1,x}$ were obtained from tables of percentage points of the incomplete beta-function (Thompson, 1941) or by direct calculation for $r = o$ and n. The confidence limits of the loglog transforms of $P_{1,x}$ and $P_{2,x}$ given in table 7 are accurate in the second decimal place.

simple as this one. Certainly the data cannot be considered to demonstrate a trend in D values calculated by the unweighted average method that is greater than the inherent bias in this method.

It must be emphasized that the anomalies in the Pflug and Esselen data are striking because fairly large numbers of tests were run. These authors correctly point out that large numbers are required for narrow confidence limits; they appear to assume that large numbers guarantee high confidence. There is no reason to assume that other experiments of this type done with fewer numbers are not subject to similar disturbances, in the absence of a demonstration of statistical control. The many pertinent biological factors are beyond the scope of this discussion.

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SUMMARY

In connection with the estimation of decimal reduction times (D values) for populations that decrease exponentially with time ("logarithmic order of death"), the calculation of D for individual times of heating from binomial data (dilution endpoint counts) gives an apparent dependence of D on time of heating. It is demonstrated that the apparent dependence arises from the bias obtained by rejecting observations where all samples show growth (short periods of heating) and where no samples show growth (long periods of heating). Preferred methods of calculation (Spearman-Kärber and loglog methods) are illustrated.

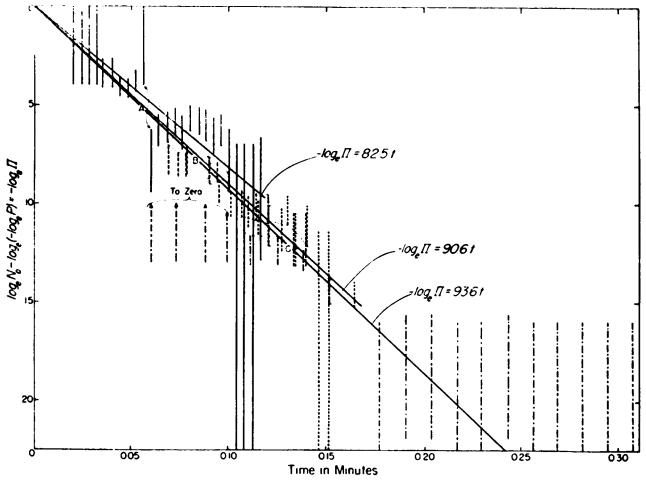

FIG. 3. Exact binomial confidence limits (99 per cent) for the data for Pflug and Esselen (1954). The diagonal lines represent the loglog estimates of k.

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